

CLAIMS

1. A DNA comprising a mutant FRT sequence having a sequence resulting from substitution of nucleotides at middle 8-bp (spacer region) in the following wild type FRT sequence (SEQ ID NO: 1) derived from yeast 2  $\mu$  DNA:

5' -GAAGTTCCTATAC 1 2 3 4 5 6 7 8  
TTTCTAGA GAATAGGAACTTC-3'  
spacer region

with nucleotide sequences selected from the group consisting of the following (1) to (4):

(1) TCTCTGGA (f2161)

(2) TCTCCAGA (f2151)

10 (3) TATCTTGA (f2262) and

(4) TTTCTGGA (f61)

wherein said mutant FRT sequence is any one of SEQ ID NOs: 2 to 5.

2. A DNA comprising a mutant FRT sequence possessing the following properties (A) and (B):

(A) causing no specific DNA recombination reaction with wild type FRT, even if FLP recombinase is present, and

(B) causing specific DNA recombination reaction with another mutant FRT sequence having an identical sequence thereto in the presence of recombinase FLP,

20 wherein the mutant FRT sequence consists of a sequence further comprising substitutions of at least one nucleotide in a region other than the spacer region in the mutant FRT sequence defined in claim 1.

3. The DNA comprising the mutant FRT sequences according to claim 1 or 2, wherein no specific DNA recombination reaction is caused with another mutant FRT sequence having a sequence different therefrom even if recombinase FLP is present.

5 4. A DNA comprising at least one wild type FRT sequence and at least one mutant FRT sequence defined in any one of claims 1 to 3.

5. The DNA according to claim 4, having a desired gene at between wild type FRT sequence and mutant FRT sequence.

10 6. A DNA comprising at least two mutant FRT sequences having different sequences in each other defined in claim 3.

7. The DNA according to claim 6, having a desired gene at between two mutant  
15 FRT sequences having different sequences in each other.

8. A cell which is transformed with the DNA of any one of claims 4 to 7.

9. A method for replacing a gene, characterized by reacting the following DNA (a)  
20 and DNA (b) in the presence of recombinase FLP, thereby obtaining the following DNA (c):

DNA (a): a DNA having a wild type FRT sequence, a gene A and a mutant FRT sequence of any one of claims 1 to 3, in this order;

DNA (b): a DNA having a wild type FRT sequence, a gene B and the same mutant FRT  
25 sequence as that of the above DNA (a), in this order;

DNA (c): a DNA in which the gene A is replaced by the gene B in the above DNA (a); wherein each of the gene A and the gene B is any gene having a sequence different from each other.

5        10.     A method for replacing a gene, characterized by reacting the following DNA (d) and DNA (e) in the presence of recombinase FLP, thereby obtaining the following DNA (f):

DNA (d): a DNA having two mutant FRT sequences of claim 3 having different sequences in each other, which are referred as mutant FRT sequence 1 and mutant FRT  
10        sequence 2, respectively, and a gene A, arranged in the order of the mutant FRT sequence 1, the gene A, and the mutant FRT sequence 2;

DNA (e): a DNA having the mutant FRT sequence 1, a gene B, and the mutant FRT sequence 2, in this order;

DNA (f): a DNA in which the gene A is replaced by the gene B in the above DNA (d);  
15        wherein each of the gene A and the gene B is any gene having a sequence different from each other.

20        11.     The method according to claim 9 or 10, characterized in that the gene B is not a functional gene.

12.     The method according to claim 9 or 10, characterized in that the gene A is not a functional gene.

25        13.     The method according to any one of claims 9 to 12, wherein DNA (a) or DNA (d) is a chromosomal DNA of a cell, and DNA (b) or DNA (e) is a plasmid DNA or a

DNA of double-stranded circular DNA virus.

14. The method according to any one of claims 9 to 12, wherein DNA (a) or DNA (d) is a chromosomal DNA of a cell, and DNA (b) or DNA (e) has a property for forming a double-stranded circular DNA by intracellular conversion.

15. The method according to any one of claims 9 to 12, wherein DNA (a) or DNA (d) is a chromosomal DNA of double-stranded DNA virus, and DNA (b) or DNA (e) is a plasmid DNA or a DNA of double-stranded circular DNA virus.

16. The method according to any one of claims 9 to 12, wherein DNA (a) or DNA (d) is a chromosomal DNA of double-stranded DNA virus, and DNA (b) or DNA (e) has a property of forming a double-stranded circular DNA by intracellular conversion.

17. The method according to claim 15 or 16, wherein the double-stranded DNA virus is adenovirus.

18. A transgenic animal carrying the DNA of any one of claims 4 to 7 on chromosomes.

19. A pharmaceutical comprising the DNA of any one of claims 4 to 7.

20. A specific DNA recombination method, characterized in that a specific DNA recombination reaction is carried out in the presence of recombinase FLP, by using two mutant FRT sequences (SEQ ID NO: 32), each resulting from substitution of G with C

at the 7th nucleotide of the spacer region in the following wild type FRT sequence

(SEQ ID NO:1) derived from yeast 2 $\mu$  DNA:

5'-GAAGTTCCTATAC	1 2 3 4 5 6 7 8	
	<u>TTTCTAGA</u>	GAATAGGAACTTC-3'
	spacer region	